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DER THE PATENT COOPERATION TREATY (PCT)

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(54) Title: ANTIVIRAL AGENTS

$$B-CH_2-O-CH(CH_2)_n-CHP(OH)_2 \quad (1)$$
$$| \quad \quad \quad \quad \| \quad \quad$$
$$R1 \quad \quad \quad O \quad R2$$

$$B-CH_2-OCH-(CH_2)_nCHR2-\overset{O}{\underset{\|}{P}}-OH \quad (1a)$$
$$\diagdown CH_2-O \diagup$$
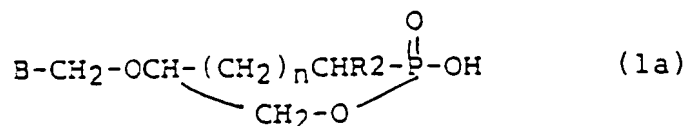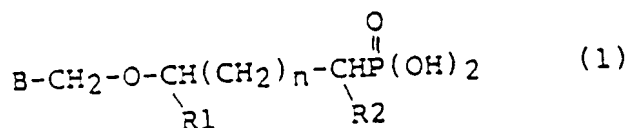
(57) Abstract

Certain open chain purine nucleoside phosphonate derivatives have antiviral activity against the Herpes group of viruses, and also show effective antiviral activity against retroviruses, including human immunodeficiency virus (HIV). These compounds have formula (1), wherein B represents a substituted or unsubstituted purine base, especially adenine or guanine and their halogenated derivatives, R1 is selected from H, methyl, hydroxymethyl, halomethyl, azidomethyl, and cyano; R2 is selected from H, methyl, hydroxymethyl, halomethyl, azidomethyl, cyano, and OH; also when R2 is OH the carbon to which it is attached may be oxidized so that the H there shown and R2 together may be =0; and n is an integer of 0-5. The compounds of the invention further include the pharmaceutically acceptable mono- and dibasic salts and the mono- and diesters of the phosphonate moiety and the acid addition salts of the murine-substituted purines. In addition, when R1 or R2 is -CH₂OH, or when R2 is OH, the acyl (1-6C) esters of these alcohols are included in the invention. Further, when n is 0, 1, or 2 and R1 is CH₂OH, the compounds of formula (1) include the cyclic forms wherein formal dehydration between R1 and one of the -OH groups on the phosphonate results in compounds of formula (1a) and their corresponding salts and esters.

-1-

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ANTIVIRAL AGENTS

Field of the Invention

10   The invention relates to nucleotide analogs
which are antiviral agents. Specifically, it relates to
acyclic purine phosphonate nucleotide analogs which are
effective against both herpes-type virus and against RNA
retroviruses such as HTLV-III (HIV).

15

Background of the Invention

There are five known herpes-type viruses which
affect human beings: herpes zoster (chicken pox), herpes
simplex virus I & II (cold sores and genital herpes),
20   cytomegalovirus (cytomegalic inclusion disease), and
Epstein-Barr virus (mononucleosis). The herpes viruses
are medium-sized viruses containing double-stranded DNA.
The nucleocapsid is about 100 nm in diameter and is
surrounded by a lipid containing envelope. The virion
25   is 150-200 nm in diameter, and permits latent infections
which last for the life span of the host even when
antibodies are present.

A very different group of virus particles is
the RNA virus group. Of particular interest within this
30   group are the retroviruses which include most tumor
virions, including human T-cell leukemia virus (HTLV
III), now designated human immunodeficiency virus (HIV).
HIV or its close relatives are believed to be the cause
of Acquired Immune Deficiency Syndrome (AIDS).

-2-

General antiviral agents are numerous.
Several nucleoside analogs, such as iododeoxyuridine and
5-E-bromovinyldeoxyuridine, are believed to act only
5   after conversion by viral thymidine kinase (but not by
host TK) to the nucleotide form, which is then converted
to the triphosphate and incorporated into the viral DNA,
thus inhibiting its replication. These analogs are
ineffective in certain herpes strains which are TK$^-$, and
10   even TK$^+$ strains resistant to certain specific members
of this class of agents have been reported (Field, H.,
et al, J Infect Disease (1981) 143:281; Hirano, A., et
al, Acta Virol (1979) 23:226). It would not be expected
that agents which work by this mechanism would be
15   effective against RNA viruses.

The antiviral agents of the present invention
are acyclic nucleotide analogs which are phosphonates
rather than phosphates. A large number of phosphonate
analogs of nucleotides are described by Engel, R., Chem
20   Reviews (1977) 11:(3)349-367. Phosphonate compounds
which are direct cyclic nucleotide analogs are also
disclosed in U.S. 3,560,478; German Patent Application
DE3,045,375VA1, published 1 July 1982; U.S. 3,446,793;
British Patent 1,243,213; German Patent Application
25   2,009,834 published 17 September 1970 and in Holman, J.,
et al, Liebigs Annalen Chem (1984) 98-107; Hampton, A.,
et al, Biochemistry (1973) 12:1730-1736; Jones, G.H., et
al, J Am Chem Soc (1968) 90:5337-5338; Montgomery, J.A.,
et al, J Med Chem (1979) 22:109-111 and British Patent
30   1,243,214. In general, in the foregoing disclosures,
the utility of the phosphonates is believed to reside in
their structure being analogous to that of the
nucleotides combined with enhanced stability due to the
phosphonate moiety. In general, it is suggested that

-3-

they are useful for whatever pharmacological purposes
the corresponding nucleotides would serve.

5   For example, Montgomery, J., et al, J Med Chem
(supra), suggest that the phosphonate analogs may be
useful as cytotoxic agents analogous to the commonly
used chemotherapeutic drug 5-fluorouracil by virtue of a
similar ability to inhibit the essential enzyme·
thymidilate synthetase, essential for DNA synthesis.
10   This enzyme is inhibited actually by the anabolic
product of 5-fluorouracil, the corresponding
monophosphate.

All of the foregoing referenced compounds
retain the furanose cyclic structure of the ribose or
15   deoxyribose. Nucleoside analogs in which the cyclic
furanose structure is replaced by an open chain are well
known antiviral agents. French Patent Application
Publication No. 2,381,781 discloses these acyclic purine
nucleosides and nucleotides; similar compounds are
20   disclosed in EPO Application Publication Nos. 0049072
and 0074306. Antiviral activity of some of these
compounds with regard to herpes simplex virus is
confirmed, for example, by Kelley, J.L., et al, J Med
Chem (1981) 34:1528-1531.
25   St. Clair, M.H., et al, Antimicrob Agents
Chemother (1980) 18:741-745, as reported in Chem
Abstracts (1981) 94:26701v, reported that virus strains
sensitive to acyclovir (9-(2-hydroxyethoxymethyl)
guanine) induced production of DNA polymerases which
30   were sensitive to its triphosphate. The same group, in
a report by Bauer, D.J., et al, Dev Antiviral Therapy
(1980) 43:51, as reported in Chem Abstracts (1981)
94:167716r disclose that acyclovir is effective in vitro
against Varicella-Zoster, Epstein-Barr virus,
cytomegalovirus, and "B" virus, and postulate that it

provides its antiviral activity by being a substrate for phosphorylation by a viral thymidine kinase, such as that specified by the herpes virus, and, in the resulting triphosphate form, inhibits herpes virus DNA polymerase.

It has now been found that a series of phosphonates containing open chain ethers in lieu of ribose or deoxyribose residues in analogs of purine nucleosides are effective as cytotoxic agents, both against herpes-type DNA virus, for example, cytomegalovirus, and against RNA virus, for example, HIV. While these analogs appear similar to acyclovir in general chemical structure, they actually are not, since the phosphonate compounds are markedly different from the nonphosphorylated acyclovir. It is also clear that the mechanism of action for the compounds of the invention in preventing proliferation of viral infection cannot be the same as that for acyclovir, since phosphorylation by viral thymidine kinase cannot occur.

After the date of the disclosure herein, publications have appeared disclosing anti-herpes activity of 9-(3-phosphono-1-propoxymethyl) guanine (EPO Publication 0173,624, published 3 May 1986) and 9-(3-phosphono-1-hydroxymethyl-1-propoxymethyl) guanine (Duke, A.E., et al., Antiviral Res (1986) 6:299-308; Prisbe, E.J., et al., J Med Chem (1986) 29:671-675).

Disclosure of the Invention

The invention provides a class of open chain alkoxymethyl nucleoside analog phosphonates which are potent antiviral agents against both herpes-type, such as cytomegalovirus, and RNA retrovirus type infections. These compounds are apparently able to penetrate the infected cell and successfully inactivate the virus.

-5-

Accordingly, in one aspect, the invention is directed to compounds of the formula:
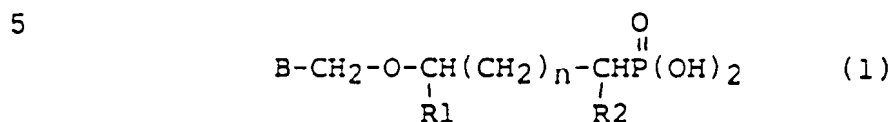
$$B\text{-}CH_2\text{-}O\text{-}\underset{\underset{R1}{|}}{CH}(CH_2)_n\text{-}\underset{\underset{R2}{|}}{CH}\overset{\overset{O}{\|}}{P}(OH)_2 \qquad (1)$$

wherein B represents a substituted or unsubstituted purine base, especially adenine or guanine and their halogenated derivatives, R1 is selected from H, methyl, hydroxymethyl, halomethyl, azidomethyl, and cyano; R2 is selected from H, methyl, hydroxymethyl, halomethyl,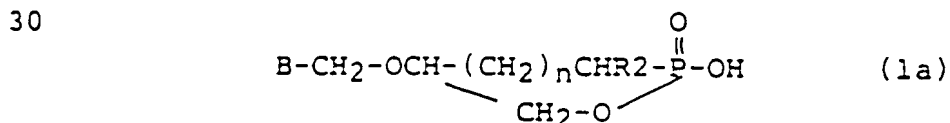 azidomethyl, cyano, and OH; also when R2 is OH the carbon to which it is attached may be oxidized so that the H there shown and R2 together may be =O; and n is an integer of 0-5. The compounds of the invention further include the pharmaceutically acceptable monoand dibasic salts and the mono- and diesters of the phosphonate moiety and the acid addition salts of the amino-substituted purines. In addition, when R1 or R2 is $-CH_2OH$, or when R2 is OH, the acyl (1-6C) esters of these alcohols are included in the invention.

Further, when n is 0, 1, or 2 and R1 is CH_2OH, the compounds of Formula 1 include the cyclic forms wherein formal dehydration between R1 and one of the -OH groups on the phosphonate results in compounds of the formula:

$$B\text{-}CH_2\text{-}O\underset{\diagdown}{CH}\text{-}(CH_2)_n CHR2\text{-}\overset{\overset{O}{\|}}{P}\text{-}OH \qquad (1a)$$
$$CH_2\text{-}O$$

and their corresponding salts and esters.

-6-

        In other aspects, the invention relates to
pharmaceutical compositions containing the compounds of
Formula 1 or 1a and to methods of treating or preventing
  5   herpes-type and RNA retrovirus infections by use of the
compounds of Formula 1 (or 1a) or these compositions.

Modes of Carrying Out the Invention

  10   A.   Description of the Compounds of the Invention
        The compounds of the invention may be in the
form of phosphonic acids, esters, or pharmaceutically
acceptable salts. In addition, the compounds may be
used as the acid addition salts of the purine base.
  15   The salts of the phosphonic acid moiety are
salts with inorganic or organic bases and may be the
mono- or dibasic salts. Salts derived from inorganic
bases include the sodium, potassium, lithium, alkaline
metal, such as magnesium and calcium salts, and salts of
  20   the transition metals or of aluminum. Salts derived
from ammonium, potassium, sodium, calcium and magnesium
are preferred. Suitable organic bases which are
nontoxic and are capable of forming salts with the
phosphonic acid moiety include various amines such as
  25   ethanolamine, triethylamine, isopropylamine, and amino
acids such as lysine and arginine.
        The phosphonic acid moiety of the compounds of
the invention may also be in the form of the mono- or
diester. The esters will be formed from alkyl,
  30   aryl-alkyl, and aryl alcohols containing 1-8 C. Alkyl
alcohols include saturated or unsaturated straight
chain, branch chain, or cyclic hydrocarbyl alcohols
which may be substituted with one or two additional
hydroxyl substituents or contain heterocyclic rings
which include N, O and/or S atoms. Such moieties

-7-

include, for example, methyl, isobutyl, n-octyl,
2-butenyl, 4-hydroxy-n-pentyl and so forth. When the
alcohol is aryl or aryl-alkyl, the ester contains a
5   phenyl substituent optionally substituted by one or two
halo, alkoxy or hydroxy residues and representative
groups of this class include the phenyl ester, the
3-hydroxyphenyl ester, the benzyl ester, and the
2-phenylethyl ester. Especially preferred are the alkyl
10   (1-6C) esters.

       Since the purine substituents represented by B
are basic, the compounds of the invention may also be
supplied as the acid addition salts which retain the
biological effectiveness and properties of the free
15   bases and which are not otherwise undesirable. These
salts may be formed from inorganic acids such as
hydrochloric, hydrobromic acid or sulfuric acid, or from
organic acids such as acetic, propionic, glycolic,
oxalic, malonic, succinic, tartaric, cinnamic, methane
20   sulfonic, p-toluene sulfonic, salicylic, and so forth.

       R1 is selected from hydrogen, methyl,
substituted methyl and cyano. Substituted methyl groups
include CH_2OH, CH_2N_3, CH_2Cl, CH_2Br, CH_2F, and CH_2I. R2
is selected from this same group, but can, in addition,
25   be OH; or when -OH, can be in oxidized form so that R2
in combination with H attached to the same C become =O.
Further, when R2 or R1 is CH_2OH, or R2 is OH, the acyl
esters (1-6C), such as the esters of acetic, proprionic,
butyric, and hexanoic acids, are also included.

30   The purine moieties of the invention are
derived either from adenine or guanine each of which has
a nucleus of the formula:

-8-
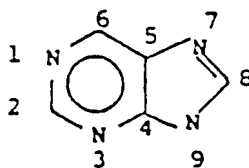
which has the numbering system shown. The numbering
system will be retained herein as shown, regardless of
substituents to the ring system. The bond at position 9
conjugates the acyclic or cyclic sugar derived moiety;
further substitutions can be made at positions 2, 6 and
8.

For guanine the substituent at position 6 is
hydroxyl (in the tautomer shown) and in adenine, $-NH_2$.
Guanine has $-NH_2$ at position 2, adenine is
unsubstituted. These and their further substituted
analogs are preferred embodiments of the compounds of
the invention. In addition, guanine or adenine analogs
having halo groups in place of the hydroxyl or amino at
position 6, such as chloro or bromo in particular, are
preferred. In general suitable substituents at
positions 2, 6 and 8 include hydroxyl, amino and halo
wherein halo is defined as fluoro, chloro, bromo, and
iodo.

Preferred values for n in the compound of
Formula 1 are 0-2.

Representative embodiments of the compounds of
the invention include:

9(3'-diethylphosphono-1'-propyloxymethyl)guanine;
9(3'-diethylphosphono-1'-propyloxymethyl)adenine;

9(3'-di-i-propylphosphono-1'-propyloxymethyl)adenine;

9(3'-di-i-propylphosphono-1'-propyloxymethyl)guanine;

9(3'-i-propylphosphono-1'-propyloxymethyl)guanine;

5

9(3'-dibutylphosphono-1'-n-butyloxymethyl)guanine;

6-chloro-9(4'-dibutylphosphono-1'-n-butyloxymethyl)
guanine;

10 6-chloro-9(4'-dibutylphosphono-1'-n-butyloxymethyl)
adenine;

6-chloro-9(5'-phosphono-1'-n-pentyloxymethyl-1'-
choromethyl)guanine;

15 6-chloro-9(5'-phosphono-1'-n-pentyloxymethyl-1'-
chloromethyl)adenine;

6-chloro-9(5'-phosphono-1'-n-pentyloxymethyl)adenine;

6-chloro-9(4'-phosphono-1'-n-pentyloxymethyl)adenine;

20 6-chloro-9(4'-phosphono-1'-n-pentyloxymethyl)guanine;

9(6'-phosphono-1'-n-hexyloxymethyl-1'-hydroxymethyl)
guanine;

8-bromo-9(6'-phosphono-1'-n-hexyloxymethyl-1'-

25 hydroxymethyl)guanine;

8-chloro-9(6'-phosphono-1'-n-hexyloxymethyl-1'-
hydroxymethyl)guanine;

9(6'-phosphono-1'-n-hexyloxymethyl-1'-hydroxymethyl)

30 adenine;

8-chloro-9(6'-phosphono-1'-n-hexyloxymethyl-1'-
hydroxymethyl)adenine;

8-bromo-9(6'-phosphono-1'-n-hexyloxymethyl-1'-
hydroxymethyl)adenine;

-10-

6-chloro-9(6'-phosphono-1'-n-hexyloxymethyl-1'-hydroxy
   methyl)adenine;

6-chloro-9(6'-phosphono-1'-n-hexyloxymethyl-6'-keto)
   guanine;

6-chloro-9(4'-phosphono-1'-n-pentyloxymethyl-1'-hydroxy
   methyl)guanine;

6-chloro-9(5'-phosphono-1'-n-pentyloxymethyl-5'-keto)
   adenine; and

6-chloro-9(4'-phosphono-1'-n-butyloxymethyl-4'-keto)
   adenine;

and their various esters and salts as set forth above.

B. **Preparation of the Invention Compounds**

Reaction Scheme 1 shows the general method to
prepare the compounds of Formula 1.

$$(R'O)_2P(O)CH(CH_2)_n\text{-}CHOH \xrightarrow[HCl]{CH_2O} (R'O)_2P(O)CH(CH_2)_nCHOCH_2Cl$$

with $R2$ and $R1$ substituents.

$$\Big/ \text{Silated B}$$

$$(R'O)_2P(O)CH(CH_2)_nCHOCH_2B$$

with $R2$ and $R1$ substituents.
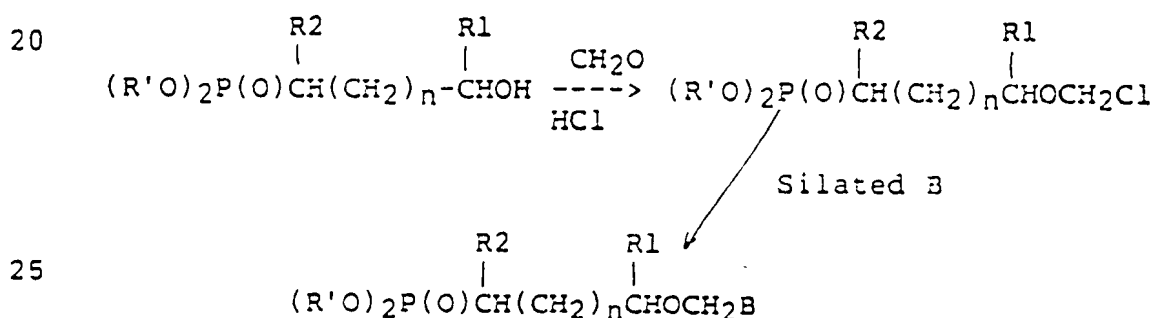
Reaction Scheme 1

As shown in Reaction Scheme 1, treatment of
the intermediate alcohol, $(R'O)_2P(O)CH(R2)(CH_2)_n$
$CH(R1)OH$, with formaldehyde in the presence of
hydrochloric acid provides the corresponding
chloromethyl ether. The purine base, B, is then
conjugated to the resulting acyclic moiety by
displacement of the chloride with purine, using, for

-11-

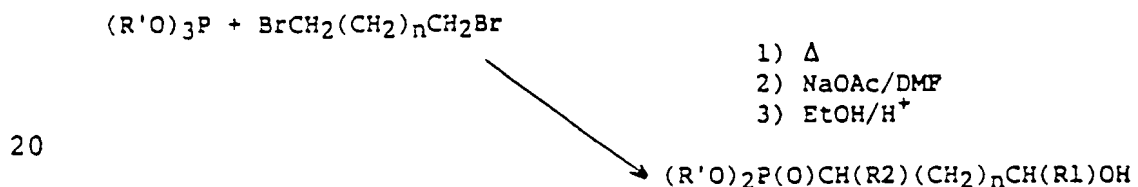example, a silated purine according to the method of
Robins, et al, Can J Chem (1982) 60:547-553.

The resulting diester is, of course, a
compound of the invention, that can be converted to the
5   free acid by a modification of the method of McKenna, et
al, Tet Letts (1977) 155.

The monoester is prepared by treatment of the
diester with aqueous 1 N sodium hydroxide, as described
by Jones and Moffatt, J Am Chem Soc (1968) 90:5337. Any
10   6-chloro group on the purine will simultaneously be
replaced by -OH.

The intermediate alcohol is obtained by
methods which depend on the nature of R1 and R2. When
R1 and R2 are either both methyl or both H, this alcohol
15   is made according to Reaction Scheme 2.

$(R'O)_3P + BrCH_2(CH_2)_nCH_2Br$

1) Δ
2) NaOAc/DMF
3) EtOH/H$^+$

20

$(R'O)_2P(O)CH(R2)(CH_2)_nCH(R1)OH$
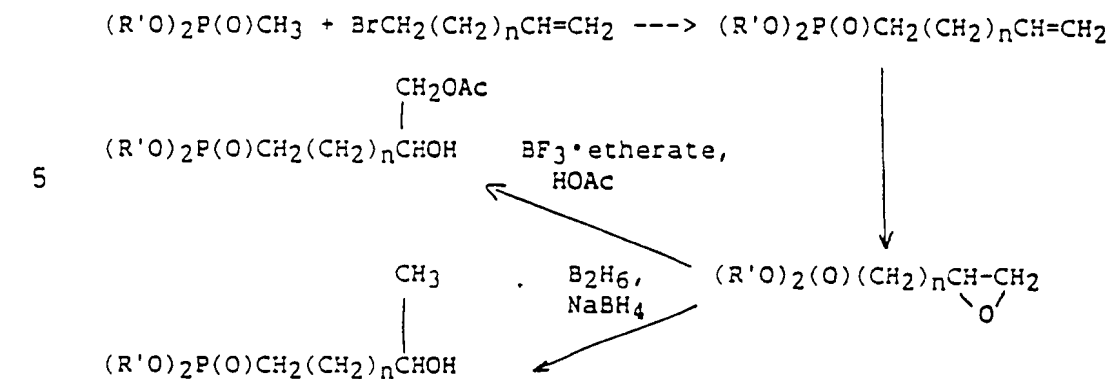
Reaction Scheme 2

Reaction Scheme 2 describes the condensation
25   of a trialkyl phosphite, wherein R' represents alkyl,
with a symmetric dibromoalkane, according to the
procedure of Eberhard and Westheimer, J Am Chem Soc
(1965) 87:253.

However, when R2 is H and R1 is hydroxymethyl
30   or methyl, the required alcohol intermediate is prepared
according to Reaction Scheme 3.

-12-

$$(R'O)_2P(O)CH_3 + BrCH_2(CH_2)_nCH=CH_2 \longrightarrow (R'O)_2P(O)CH_2(CH_2)_nCH=CH_2$$

$$\underset{\displaystyle (R'O)_2P(O)CH_2(CH_2)_nCHOH}{\overset{\displaystyle CH_2OAc}{|}} \quad \underset{HOAc}{BF_3 \cdot etherate,}$$

$$(R'O)_2(O)(CH_2)_n\underset{\diagdown O \diagup}{CH-CH_2}$$

$$\underset{\displaystyle (R'O)_2P(O)CH_2(CH_2)_nCHOH}{\overset{\displaystyle CH_3}{|}} \quad . \quad \underset{NaBH_4}{B_2H_6,}$$
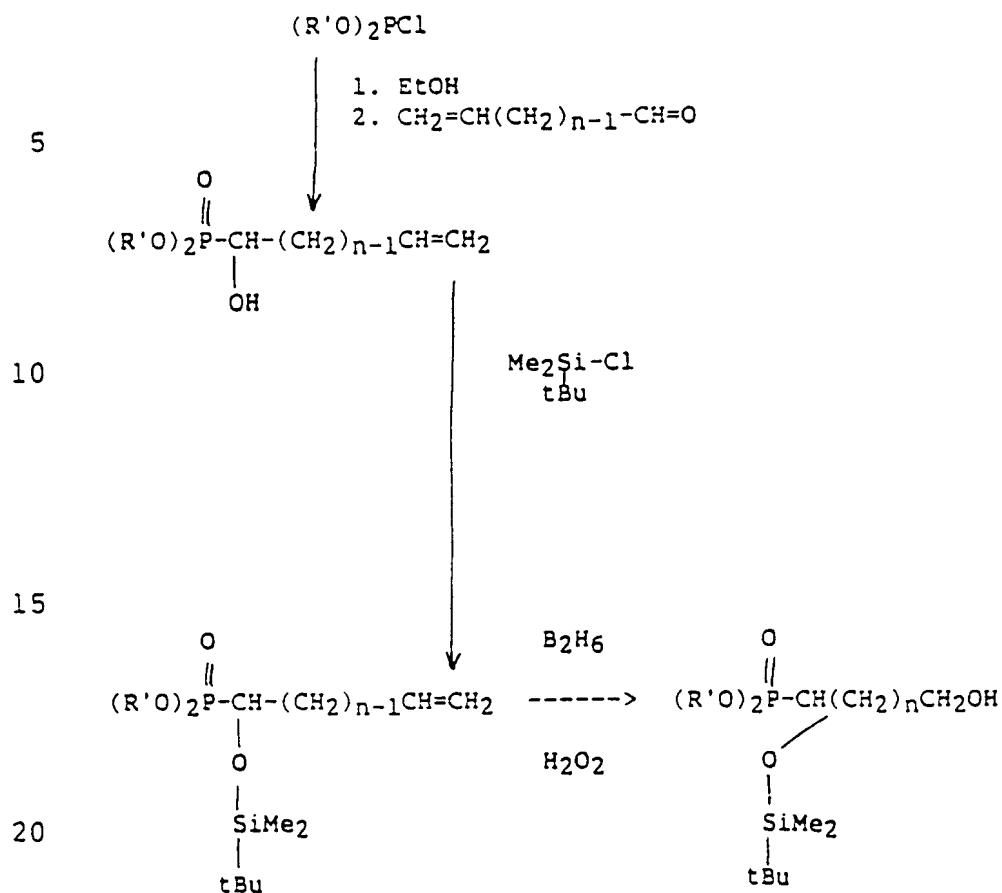
Reaction Scheme 3

The initial condensation of Reaction Scheme 3
is according to the method of Savignac, et al., <u>Synth</u>
<u>Comm</u> (1979) <u>9</u>:487. Treatment of the resulting
unsaturated butyl phosphonate with meta-peroxybenzoic
acid, as shown, gives the epoxide. The epoxide can then
be cleaved with BF3 etherate in glacial acetic acid to
obtain an acetoxymethyl intermediate for R1 as
hydroxymethyl; or can be reduced with diborane and
sodium borohydride to yield R1 as methyl.

For embodiments wherein R1 is H and R2 is OH,
Reaction Scheme 4 is employed to form the intermediate
alcohol, derivatized to the silyl ether.

-13-

(R'O)$_2$PCl

    1. EtOH
    2. CH$_2$=CH(CH$_2$)$_{n-1}$-CH=O

5

$$
\underset{\underset{\displaystyle OH}{|}}{(R'O)_2\overset{\displaystyle O}{\overset{\displaystyle \|}{P}}-CH-(CH_2)_{n-1}CH=CH_2}
$$

10

Me$_2$Si-Cl
tBu

15

$$
\underset{\underset{\displaystyle SiMe_2}{\underset{\displaystyle |}{\underset{\displaystyle tBu}{|}}}}{\underset{\displaystyle O}{\underset{\displaystyle |}{(R'O)_2\overset{\displaystyle O}{\overset{\displaystyle \|}{P}}-CH-(CH_2)_{n-1}CH=CH_2}}}
$$

B$_2$H$_6$
------->
H$_2$O$_2$

$$
\underset{\underset{\displaystyle SiMe_2}{\underset{\displaystyle |}{\underset{\displaystyle tBu}{|}}}}{\underset{\displaystyle O}{\underset{\displaystyle /}{(R'O)_2\overset{\displaystyle O}{\overset{\displaystyle \|}{P}}-CH(CH_2)_nCH_2OH}}}
$$

20

Reaction Scheme 4

25   In this process, the intermediate phosphonate is prepared as described by Gazizov, T., et al, <u>Gen Chem USSR</u> (1977) <u>47</u>:2465, and then derivatized with alkyl chlorosilane before functionalization of the alkene to form the desired alcohol.

30   C.   <u>Interconversion of Salt and Ester Derivatives</u>
    The compounds of Formulas 1 and 1a may be prepared in their salt or ester forms, and these esters or salts can be reconverted to the free acid or free purine base.

-14-

Phosphonic Acid Salts

If desired, the free phosphonic acid may be
converted to the mono- or dibasic salt form by treatment
5   with an appropriate base. These salts are prepared by
treating the corresponding free acids with at least one
or at least two molar equivalents of a pharmaceutically
acceptable base as set forth above. The reaction is
conducted in water, alone or in combination with an
10   inert, water miscible organic solvent at a temperature
of from 0°C-100°C, preferably room temperature. Typical
inert, water miscible organic solvents include methanol,
ethanol and dioxane. The stoichiometry of the resulting
salt is dependent on the stoichiometry of the reaction
15   components.

The salts can be reconverted to the phosphonic
acid by standard procedures, e.g. by neutralizing with
an acid resin or, less preferably, with an organic acid.

20   ## Acid Addition Salts

Alternatively, acid addition salts of the
purine moiety may be prepared by reacting a compound of
the invention with an acid such as the organic or
inorganic acids exemplified above. The free base is
25   dissolved in an polar organic solvent such as methanol
or ethanol and the acid is added at a temperature of
0-100°C, preferably at room temperature. The resulting
salt either precipitates or may be brought out of
solution by addition of a less polar solvent.
30   Conversely, the acid addition salt is
reconverted to the free base by treating with suitable
base.

-15-

Phosphonic Acid Esters

Desired esters of the phosphonic acid moiety
may be prepared by transesterification of the phenyl
esters using methods illustrated by Jones and Moffatt,
5   J Am Chem Soc (1968) 90:5337. Although the examples
below result in diethyl esters, the corresponding phenyl
esters could be prepared by analogous methods by
substituting phenyl phosphonate ester starting materials
for the ethyl phosphonate ester starting materials
10   illustrated.

The esters can be reconverted to the free
acids by hydrogenolysis of the benzyl or hydrolysis of
trimethyl silyl esters. These esters may be prepared by
transesterification as above described.
15

Esters of R1/R2 Alcohols

These esters can result directly from the
synthetic pathways illustrated below.

20   D.   Utility and Administration

The compounds of this invention (including the
physiologically acceptable salts and esters thereof)
have antiviral activity against herpes virus forms and
against RNA retroviruses. The compounds are
25   conveniently formulated into pharmaceutical preparations
composed of one or more of the compounds in association
with a pharmaceutically acceptable carrier. Remington's
Pharmaceutical Sciences, latest edition, by E.W. Martin
(Mack Publ. Co., Easton, PA) discloses typical carriers
30   and methods of preparation known in the art.

The compounds may be administered topically,
orally, parenterally (e.g., intravenously), by
intramuscular injection, or by intraperitoneal
injection, or the like, depending upon the nature of the

-16-

viral infection being treated. For internal infections
the compositions are administered orally or parenterally
at dose levels of about 0.1 to 300 mg/kg, preferably 1.0
to 30 mg/kg of mammal body weight and can be used in
5   humans in a unit dosage form administered one to four
times daily in the amount of 1-250 mg per unit dose.
            For oral administration, fine powders or
granules may contain diluting, dispersing, and/or
surface active agents, and may be presented in water or
10   in a syrup, in capsules or sachets in the dry state, or
in a nonaqueous solution or suspension wherein
suspending agents may be included, in tablets wherein
binders and lubricants may be included, or in a
suspension in water or a syrup. Where desirable or
15   necessary, flavoring, preserving, suspending,
thickening, or emulsifying agents may be included.
Tablets and granules are preferred oral administration
forms, and these may be coated.
            Alternatively, for topical infections, e.g.,
20   mouth and skin, the compositions are preferably applied
to the infected part of the body of the patient
topically as an ointment, cream, aerosol, or powder,
preferably as an ointment or cream. The compounds may
be presented in an ointment, for instance with a water
25   soluble ointment base, or in a cream, for instance with
an oil in water cream base in a concentration of from
about 0.01 to 10%, preferably 0.1 to 7%, most preferably
about 0.5% w/v. Additionally, viral infections of the
eye, such as Herpetic keratitis, may be treated by use
30   of a sustained release drug delivery system as is
described in the art.
            The exact regimen for administration of the
compounds and compositions disclosed herein will
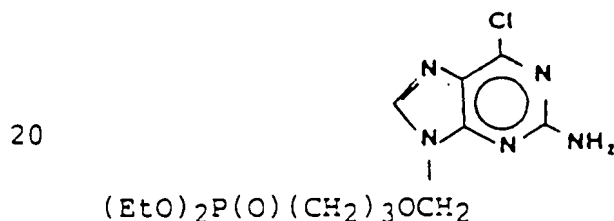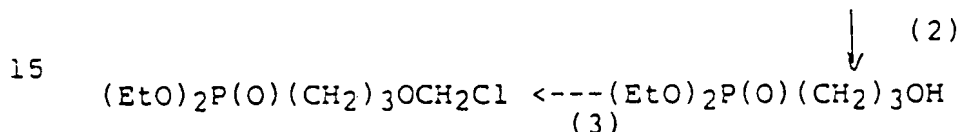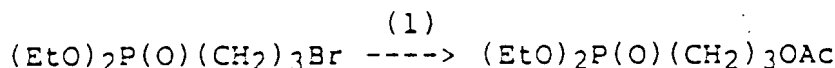necessarily be dependent upon the needs of the

-17-

individual subject being treated, the type of treatment, and, of course, the judgment of the attending practitioner.

5   E.   Examples
          The invention will be further described by the following nonlimiting examples.

Example 1
Preparation of 6-Chloro-9(3'-diethyl
phosphono-1'-propyloxymethyl) Guanine
$$(EtO)_2P(O)(CH_2)_3Br \xrightarrow{(1)} (EtO)_2P(O)(CH_2)_3OAc$$

$$\Big\downarrow (2)$$

$$(EtO)_2P(O)(CH_2)_3OCH_2Cl \xleftarrow{(3)} (EtO)_2P(O)(CH_2)_3OH$$

$(EtO)_2P(O)(CH_2)_3OCH_2$

          (This is not precisely a guanine derivative, since the -OH at position 6 is not present, and "B" should properly be called 2-amino-6-chloropurine; however, 6-chloroguanine is conventionally used.)
          Diethyl-3-hydroxypropylphosphonate (steps 1 and 2) is prepared from diethyl-3-bromopropyl-phosphonate. Diethyl-3-bromopropylphosphonate (12.0 g, 46 mmol), prepared by the method of Eberhard, A., et al, J Am Chem Soc (1965) 87:253-260) was stirred with 12.0 g NaOAc·3H$_2$O in 125 ml DMF heated in a steam bath. The reaction was evaporated to dryness in vacuo after 2 hours and partitioned between H$_2$O and EtOAc, extracting

the aqueous layer five times. The ethyl acetate extract
was washed once with brine, dried with Na_2SO_4, filtered,
and evaporated to dryness in vacuo to yield 9.8 g light
yellow oil (89%). ^{1}H NMR ($CDCl_3$) δ 1.3 (tr, 6 H),
1.5-2.0 (m, 4 H), 2.03 (s, 3 H), 4.1 (dq, 6 H); thin-
layer chromatography on SiGF developed with 2:1
EtOAc:CH_2Cl_2 gave R_f 0.30. The isolated diethyl-3-
acetoxypropylphosphonate (9.8 g, 41 mmol) in 200 ml abs.
EtOH was stirred with 30 ml Dowex 50 (H^+) which had been
rinsed three times each with H_2O and EtOH. After 4-1/2
days at room temperature, another 10 ml of similarly
prepared resin was added. Six hours later, the reaction
was filtered and evaporated in vacuo. The quantitative
yield of yellow oil was purified by dry column
chromatography on 400 g silica packed in a 2.75 inch
flat diameter nylon tube. The column was eluted with
1:9 MeOH:EtOAc and the appropriate fractions were cut
and slurried with 1:1 MeOH:EtOAc. Filtration and
evaporation in vacuo afforded 5.33 g (66%) pale yellow
oil, which is the diethyl ester of 3-hydroxypropyl
phosphonic acid. ^{1}H NMR ($CDCl_3$, D_2O): δ 1.30 (tr, 6 H),
1.60-2.08 (m, 4 H), 3.67 (tr. 2 H), 4.13 (cq, 4 H);
thin-layer chromatography on SiGF developed with 1:9
MeOH:EtOAc gave an R_f of 0.57.

        Conversion to diethyl-3-chloromethoxypropyl
phosphonate was according to the procedure of Kelley,
J.L., et al, J Med Chem (1981) 24:1528-1531.

        To 0.5 g (2.95 mmol) 2-amino-6-chloropurine,
silated and treated with Hg(CN)$_2$ according to the
procedure of Robins, M.J., et al, Can J Chem (1982)
60:547-553, in 40 ml benzene, was added a solution of
2.68 mmol diethyl-3-chloromethoxypropylphosphonate
prepared as above. The reaction was refluxed for 2
hours, cooled, and 400 ml $CHCl_3$ was added. The organic

-19-

phase was washed successively with 80 ml each of aqueous
saturated NaHCO3 and 1 M aqueous KI. The organic
solution was dried over Na2SO4, filtered, and evaporated
to 790 mg of yellow gum. A portion of this crude
5   material was used to conduct hydrolysis experiments. The
remaining material was chromatographed on a silica
column. A solution of 574 mg of the crude reaction
product was placed on 20 g silica packed in a column
using 5:3 EtOAc:nPrOH. Elution with the same mixed
10   solvent afforded sixteen fractions of 10-20 ml each.
Fractions 7-12 were combined to yield 258 mg of a
colorless oil which spontaneously crystallized.
Trituration in $CH_2Cl_2 \cdot Et_2O$ afforded two crops of white
solid (207 mg), mp 109-110° (28%). A yield of 46% of
15   the diethyl ester was obtained from a reaction performed
on 45.2 mmol of the starting purine. Anal
($C_{13}H_{21}ClN_5O_4P$) C,H,N; UV λ_{max} (ϵ): pH 1, 246 (ϵ 6600),
310 (7200); pH 7, 247 (6800), 308 (7400); pH 11 247
(6600), 308 (7100); mass spectrum: m/e 377 (M^+); ^{1}H NMR
20   ($CDCl_3$): δ 1.3 (tr, 6 H), 1.52-2.18 (m, 4 H), 3.58 (tr,
2 H), 4.09 (dq, 4 H), 5.48 (s. with broad base, 4 H),
7.89 (s, 1H). Thin-layer chromatography on SiGF
developed with 5:3 EtOAc:nPrOH gave R_f 0.40.

25   Example 2
                        Preparation of
           9(3'-ethylphosphono-1'-propyloxymethyl)guanine

          A. 6-Chloro-9(3'-diethylphosphono-1'-
30   propyloxymethyl) guanine (75 mg; 0.2 mmol) prepared as
in Example 1 was combined with 5 ml 1 N aqueous NaOH and
refluxed 1 hour. The cooled reaction was neutralized
with Dowex 50X8 (pyridinium form) and filtered, rinsing
liberally with water. The solution was partially

-20-

evaporated to remove pyridine and was then lyophilized.
The orange-colored residue (74 mg) was redissolved in
H_2O and centrifuged to remove insoluble material. The
decanted solution (2 ml) was chromatographed on a 0.9 x
46 cm column of Whatman DE-52 Cellulose, HCO_3 form,
using a linear gradient of one liter each H_2O and 0.2 M
NH_4HCO_3 after an initial H_2O elution. Fractions (7 ml
each) 43-47 yielded 25 mg (36%) of fluffy white solid
after three lyophilizations. Electron impact mass
spectrum (TMS derivative) showed m/e 547 (M^+ of TMS
derivative); chemical ionization mass spectrum (TMS
derivative)showed m/e 548 (M^+ + H of TMS_3 derivative).
^1H NMR (D_2O) showed δ 1.19 (tr, 3 H), 1.4-1.9 (m, 4 H),
3.59 (tr, 2 H), 3.90 (dq, 2 H), 5.47 (s, 2 H), 8.2 (brs,
1 H). Thin-layer chromatography behavior on SiGF:
R_f=0.40 when developed with 7:3 CH_3CN:0.1 N aqueous
NH_4Cl. Material obtained from passage through a column
of Dowex 50X8 (H^+) had a formula of ($C_{11}H_{18}N_5O_5P \cdot H_2O$)
Calc: C-37.82% H-5.77%, N-20.05. Found: C-38.27%,
H-5.84%, N-19.65%. A UV spectrum was run on the
material and showed UV λ_{max} (ε): pH 1, 255 (11,900), 278
shoulder; pH 7, 252 (12,800) 271 shoulder; pH 11, 257
(10,700) 267 shoulder.

(The hydrolysis to the monoester.
simultaneously effects dehalogenation of the purine
ring.)

B. In an improved procedure, a mixture of 6-
chloro-9(3'-diethylphosphono-1'-propyloxymethyl) guanine
(4.27g, 11.3 mmol) and 1N aqueous NaOH (200ml) was
gently refluxed for 1 hour. The cooled reaction was
neutralized and desalted with Dowex 50 X 8 (pyridinium
form). The resin was removed by filtration, rinsing
thoroughly with water. The aqueous filtrates were

-21-

partially evaporated to remove pyridine and then
lyophilized. A solution of the residue (3.68g) in H_2O
(30 ml, pH 8) was chromatographed on a bed of
DEAE-Cellulose (Whatman DE 52, HCO_3^- form, 2.5 x 100
5   cm). After an initial water wash to constant baseline,
the column was eluted with a linear gradient of two
liters each H_2O and 0.1N NH_4HCO_3. Fractions (20 ml
each) 89-139 were combined and lyophilized to constant
weight (2.25g).

10   At 0°C, a solution of this material in a
minimum amount of H_2O plus an equal amount of EtOH was
treated with cold 1N HCl to pH 3.5 to yield 1.31g (34%)
of the title compound as a fine white solid. Electron
impact mass spectrum (TMS derivative) showed m/e 547 (M^+
15   of TMS_3 derivative); chemical ionization mass spectrum
(TMS derivative) showed m/e 548 (M^++H of TMS_3
derivative); chemical ionization mass spectrum on
underivatized compound gave m/e 332 (M^++H). Further
characterization by ^1H NMR (DMSO-d_6) showed δ 1.17 (tr,
20   3H), 1.3-1.8 (m, 4H), 3.48 (tr, 2H), 3.85 (dq, 2H), 5.30
(s, 2H), 6.46 (br s, 2H), 7.79 (s, 1H). An ultraviolet
spectrum showed UV λ_{max} (ε): pH 1, 254 (12,800) 275
shoulder; pH 7, 252 (13,200) 272 shoulder; pH 11, 257
(11,400) 267 shoulder. The material had a formula of
25   ($C_{11}H_{18}N_5O_5P$ · 1/2 H_2O). Calc: C-38.82%, H-5.63%,
N-20.58%. Found: C-38.52%, H-5.90%, N-20.34%. Thin
layer chromatography behavior on SiGF: R_f=0.40 when
developed with 7:3 CH_3CN:0.1 N NH_4Cl.

30   Example 3
        Conversion of the Monoester to Free Acid:
        9(3'-Phosphono-1'-propyloxymethyl)guanine
             In a modification of the procedure of McKenna
et al, Tet. Letters, (1977) 155, to a stirring mixture

-22-

of 200 mg (0.57 mmol) 9(3'-ethylphosphono-l'-propyloxy
methyl)guanine in $CHCl_3$ (8.3 ml) and hexamethyldi-
silazane (8.3 ml) at room temperature was added 0.61 ml
(4.60 mmol) bromotrimethylsilane. After 72 hours, the
5   mixture was evaporated to dryness, slurried with H_2O,
and filtered, rinsing with acetone. A white solid (125
mg) was obtained. ^{1}H NMR indicated no ethyl groups, and
thin-layer chromatography on SiGF (7:3 CH_3CN: 0.1 N
NH_4Cl) revealed a single spot at Rf 0.20. The sodium
10   salt was generated from Dowex 50 x 2 (Na^+) to yield 114
mg (58%) white solid after lyophilization. ^{1}H NMR
($DMSO \cdot d_6$ + D_2O) showed δ 1.68 (m, 4H), 3.60 (tr, 2H),
5.48 (s, 2H), 7.96 (s, 1H). An ultraviolet spectrum
showed λ_{max} (ε): pH 1, 256 (10,800) 278 shoulder; pH 7,
15   256 (11,800) 270 shoulder; pH 11, 256 (10,200) 268
shoulder. Microanalysis afforded the formula
($C_9H_{13}N_5O_5P \cdot Na \cdot H_2O$) Calc: C-31.49%, H-4.41%,
N-20.41%, P-9.02%. Found: C-31.52%, H-4.55%, N-20.10%,
P-8.21%; mass spectrum m/e 591 (M^+ for TMS_4 derivative).
20

25

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-23-

Example 4

Preparation of

6-Chloro-9(7'-diethylphosphono-1'-heptoxymethyl)guanine

$(EtO)_2P(O)(CH_2)_7O \; CH_2$
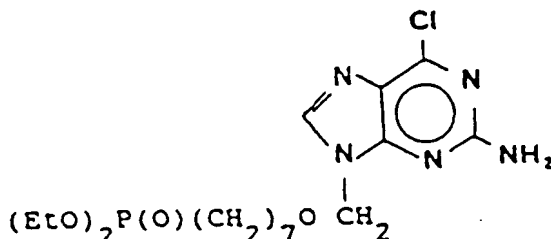
Diethyl7-chloromethoxyheptylphosphonate was
prepared from 1,7-dibromoheptane and triethylphosphite
in a manner analogous to that of Example 1. It was
reacted with silated 2-amino-6-chloropurine, and
mercuric cyanide as described for the preparation of
6-chloro-9(3-diethylphosphono-1-propyloxymethyl)guanine
in Example 1 to give 32% of product as a colorless gum.
UV λ_{max} (ϵ): pH 1 -246 nm (6970), 310 nm (7190); pH 7
-247 nm (6620), 310 nm (7180); pH 11 -246 nm (6660), 309
nm (7140); ^{1}H NMR (CDCl$_3$) δ 1.1-1.9 (m, 18 H), 3.48 (t,
2 H), 4.10 (dq, 4 H), 5.47 (s, 2 H), 5.88 (s, 2 H), 7.93
(s, 1 H). Thin-layer chromatography on SiGF gave R$_f$
0.15 using ethyl acetate:ethanol (100:1); analysis shows
$C_{17}H_{29}ClN_5O_4P \cdot H_2O$.

Example 5

Conversion to the Monoester:

9(7'-ethylphosphono-1'-heptyloxymethyl)guanine

6-Chloro-9(7'-diethylphosphono-1-heptyloxy
methyl) guanine of Example 4 was hydrolyzed by refluxing
1 N aqueous sodium hydroxide for 4 hours and isolated in
30% yield as described for the preparation of 9-(3'-
ethyl phosphono-1'-propyloxymethyl)guanine (Example 2).
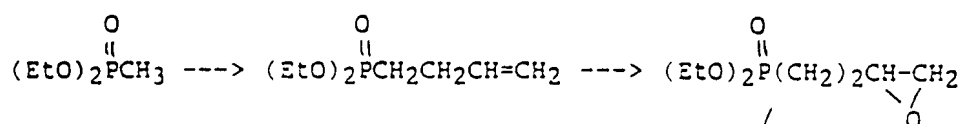It had R$_f$ 0.5 on SiGF using acetonitrile:0.1 N aqueous

ammonium chloride, (7:3) . Proton NMR (D$_2$O): δ=1.1 1.5
(m, 15 H), 3.5 (t, 2 H), 3.90 (dq, 2 H), 5.45 (s, 2 H).
UV λ$_{max}$ (ε): pH 7, 252 (12,200). Mass spectrum m/e 603
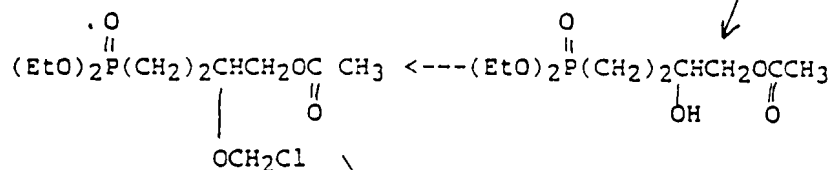(M$^+$ for TMS$_3$ derivative).

<div align="center">

Example 6
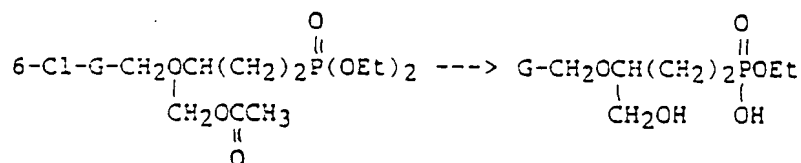### Preparation of 9(3'-ethylphosphono-1'-hydroxymethyl-1'-propyloxymethyl)guanine

</div>

$$(EtO)_2\overset{\overset{O}{\|}}{P}CH_3 \longrightarrow (EtO)_2\overset{\overset{O}{\|}}{P}CH_2CH_2CH=CH_2 \longrightarrow (EtO)_2\overset{\overset{O}{\|}}{P}(CH_2)_2CH-CH_2 \overset{}{\underset{O}{\diagdown /}}$$

$$(EtO)_2\overset{\overset{.O}{\|}}{P}(CH_2)_2\underset{\underset{OCH_2Cl}{|}}{C}HCH_2O\overset{\overset{}{}}{\underset{\underset{O}{\|}}{C}}CH_3 \longleftarrow (EtO)_2\overset{\overset{O}{\|}}{P}(CH_2)_2\underset{\underset{OH}{|}}{C}HCH_2O\overset{\overset{}{\underset{O}{\|}}}{C}CH_3$$

silated
6-chloroguanine (6-Cl-G)

$$6\text{-Cl-G-}CH_2O\underset{\underset{\underset{O}{\|}}{CH_2OCCH_3}}{\overset{}{C}H(CH_2)_2}\overset{\overset{O}{\|}}{P}(OEt)_2 \longrightarrow G\text{-}CH_2O\underset{\underset{CH_2OH}{|}}{C}H(CH_2)_2\overset{\overset{O}{\|}}{P}\underset{\underset{OH}{|}}{O}Et$$

A. Preparation of Diethyl-3,4-epoxybutanephosphonate.
Diethyl-3,4-epoxybutanephosphonate was
synthesized according to the procedure of Savignac et
al., Syn Comm (1979) 7:487. In several syntheses,
oxidation of the intermediate olefin compound produced a
contaminating white solid which was removed by
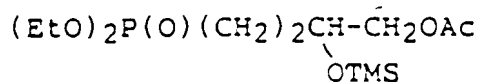filtration of the crude, neat reaction product and

-25-

rinsing the solid with ice cold CH_2Cl_2. Evaporation of
the filtrate and distillation of the residue gave the
epoxide in 75% yield.

5   B.   Preparation of Diethyl 3-hydroxy-4-acetoxy butyl
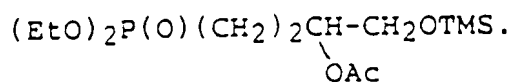   phosphonate.
          To a stirring solution of 2.51 g (12 mmol)
     diethyl-3,4-epoxybutanephosphonate in glacial acetic
     acid (10 ml) at room temperature in a dry, inert
10   atmosphere was added 0.2 ml BF_3 etherate. After
     stirring 1 hour, H_2O (20 ml) was added; the reaction was
     stirred 10 minutes and evaporated in vacuo. H_2O was
     twice added and reevaporated to azeotrope HOAc. The
     residual syrup was partitioned between saturated aqueous
15   $NaHCO_3$ (30 ml) and Et_2O (20 ml). The aqueous phase was
     extracted with $CHCl_3$ (3 x 20 ml). The $CHCl_3$ extract was
     washed once with H_2O (10 ml), dried (Na_2SO_4), filtered,
     and evaporated to obtain 2.15 g (67%) colorless oil. ^1H
     NMR ($CDCl_3$ + D_2O) spectral data indicated this material
20   to be 85% pure as measured by the ratio of the $-OCOC\underline{H}_3$
     singlet at 2.1δ to the $(C\underline{H}_3CH_2O)_2PO$-triplet at 1.32δ.
     GC/MS data on the TMS derivative indicated one major
     component whose fragmentation pattern contained an
     extremely intense absorbance at m/e 267 with only small
25   absorbance seen at m/e 237. This information is
     supportive of the structure for the desired isomer

     $(EtO)_2P(O)(CH_2)_2C\overline{H}-\overline{C}H_2OAc$
                            \
                            OTMS
30

-26-

versus undesired

$(EtO)_2P(O)(CH_2)_2CH-CH_2OTMS.$
                           \
                          OAc

C.   <u>Preparation of 6-Chloro-9(3'-diethylphosphono-1'-</u>
<u>acetoxymethyl-1'-propyloxymethyl)guanine.</u>

           6-Chloro-9(3'-diethylphosphono-1'-acetoxy
methyl-1'-propyloxymethyl)guanine was prepared by
coupling 112 mmol silated 6-chloroguanine with 108 mmol
diethyl-3-chloromethoxy-4-acetoxybutylphosphonate ('H
NMR: doublet at 5.54δ for -OCH₂Cl, prepared analogously
to Example 1); according to the procedure of Example 2
for 6-chloro-9(3'-diethyl phosphono-1'-propyloxy-
methyl)guanine. The reactions remained at room
temperature 1 week after a 3 hour reflux period. Due to
the persistence of TMS groups after the usual workup
procedure in earlier syntheses, EtOH (400 ml) was added
to the reaction and stirred 1 hour before the workup was
begun. The crude yellow gum obtained in 72% yield
(34.9 g) was purified by flash chromatography on 330 g
adsorbent (Baker silica for flash chromatography) by
stepwise elution with 2,5, and 8% EtOH in CHCl₃.
Combination of the appropriate fractions yielded 7.95 g
(16%) 6-chloro-9(3'-diethylphosphono-1'-acetoxymethyl-
1'-propyloxymethyl) guanine as a light yellow oil.
Thin-layer chromatography on SiGF (9:1 CH₂Cl₂· EtOH)
showed a single spot at Rf 0.61. ¹H NMR (CDCl₃ + D₂O):δ
1.30 (tr), 1.45-2.10 (m), 3.58 (d), 3.86-4.27 (m), 5.50
(assym d), 7.84 (d). Integration indicates that the
aromatic to -OCH₂ protons are present in a 1:2 ratio and
that protons upfield of 5.50 δ (-OCH₂N-) are present in
80% excess. An ultraviolet spectrum showed λmax (EtOH)

-27-

248, 311; TOD_{248} 14.4/mg (TOD_{248} 16.9/mg for 6-chloro-
9(3'-diethylphosphono-1'-propyloxymethyl)-guanine from
Example 1.

5   D. Conversion to the Monoester and Hydrolysis:
         9(3'-ethylphosphono-1'-hydroxymethyl-1'-propyloxymethyl)
         guanine
              The hydrolysis of 7.95 g (17.7 mmol)
         6-chloro-9(3'-diethylphosphono-1'-acetoxymethyl-1'-
10   propyloxymethyl)guanine with 1 N aqueous NaOH (130 ml)
         was conducted as in Example 3B for the synthesis of
         9(3'-ethyl phosphono-1'-propyloxymethyl)guanine.
         Material isolated from DEAE-Sephadex (2.5 x 97 cm A-25,
         HCO_3^-) chromatography required purification on a second
15   similar column again using a linear gradient of H_2O and
         0.1 N NH_4HCO_3 (2.0 liters each). Examination of
         fractions by HPLC (Vydac TP21854, 1% CH_3CN in 0.0125N
         triethylammonium formate, pH 3, 2 ml/min, 252λ), of
         ultraviolet spectra and of the total optical density
20   (TOD^{pH7}_{252}) relative to the weight obtained after
         several lyophilizations allowed one to obtain two pure
         fractions of 9(3'-ethylphosphono-1'-hydroxymethyl-1'-
         propyloxymethyl) guanine (1.0 g each, 29%, 36
         TOD^{pH7}_{252}/mg), one of which contained an isomer ratio of
25   3:2 by HPLC and the second, an isomer ratio of 1:1.
         Microanalysis of the latter sample gave the formula
         ($C_{12}H_{20}N_5O_6P \cdot 1/4\ NH_3 \cdot 1\ H_2O$). Calc: C-37.57%,
         H-5.98%, N-19.17%. Found: C-37.47%, H-5.50%, N-19.16%.
         UV λ_{max} (ε): pH 1, 256 (12,400) 276 shoulder; pH 7, 252
30   (13,500) 270 shoulder; pH 11, 256 (11,800) 266 shoulder.
         ^1H NMR (D_2O):δ 1.22 (d tr, 3H), 1.35-1.88 (m, 4H),
         3.48-4.0 (m, 5H), 5.52 (d, 2H), ~7.9 (very broad
         singlet, 1H). Thin-layer chromatography on SiGF: Rf
         0.32 (7:3 CH_3CN:0.1 N NH_4Cl).

-28-

Example 7
Preparation of 9(3'-Phosphono-1'-hydroxymethyl-
1'-propyloxymethyl)guanine

5   9(3'-Ethyl phosphono-1'-hydroxymethyl-1'-
propyl oxymethyl)guanine (204 mg, 0.52 mmol of the
partial ammonium salt) was treated with bromotrimethyl
silane (0.8 ml, 6.13 mmol) in a procedure similar to the
synthesis of 9(3'-phosphono-1'-propyloxymethyl) guanine
10   of Example 4. To aid in solubilization of the starting
material in the reaction medium, 1,5-diazabicyclo[5.4.0]
undec-5-ene (0.078 ml, 0.52 mmol) was added. After
stirring overnight at room temperature, the mixture was
evaporated to dryness. The residue was dissolved
15   readily in 3 ml H_2O and was treated with several drops
of 1 M HCl to acidity and reevaporated. Trituration
with EtOH followed by filtration yielded 161 mg
off-white solid (93%). Thin-layer chromatography on
SiGF (7:1:2, iPrOH:NH$_4$OH:H$_2$O) gave Rf 0.18. UV λ_{max}:
20   pH 1, 256, 278 shoulder; pH 7, 252, 272 shoulder; pH 11,
255, 268 shoulder. ^{1}H NMR (D$_2$O) δ:1.55-1.85 (m, 4H),
3.5-3.95 (m, 3H), 5.58 (d), 7.95 (br s, 1H). Mass
spectrum m/e 693 (M$^+$ for TMS$_5$ derivative).

25   ## Example 8
Preparation of 9(3'-phosphono-1'-
hydroxymethyl-1'-propyloxymethyl)guanine cyclic ester
In a procedure similar to that of Khorana,
H.G., et al in J Am Chem Soc (1961) 82:698, a mixture of
30   0.011 g (0.033 mmol) 9(3'-phosphono-1'-hydroxymethyl-1'
-propyloxymethyl)guanine (Example 9) in 6 ml dry
pyridine was treated with 0.010 g (0.034 mmol)
N',N'-dicyclohexyl-4-morpholine carboxamidine at reflux.
After solution was complete, a hot solution of 0.012 g

-29-

(0.058 mmol) dicyclohexylcarbodiimide in 4 ml pyridine
was added in a single portion, and the solution was
heated at gentle reflux for 2-1/2 hours. The reaction
was evaporated to dryness. The residue was triturated
in H_2O and filtered. The filtrate was evaporated to
dryness, then solubilized in MeOH. Thin-layer
chromatography on SiGF showed a single spot at R_f=0.70
using iPrOH:NH_4OH:H_2O (7:1:2) R_f=0.41 using CH_3CN:0.01 N
NH_4Cl (7:3), and R_f=0.78 using MeOH. UV λ_{max}: pH 7,
251, 275 shoulder. Mass spectrum m/e 531 (M^+ for TMS_3
derivative).

Example 9
Preparation of 8-Bromo-9 (3' ethylphosphono-1'-Propyloxymethyl) guanine

A stirring mixture of 97 mg (0.286 mmol)
9(3'-ethylphosphono-1'-propyloxymethyl) guanine,
prepared in Example 2 in H_2O (5 ml) was treated with a
saturated Br_2 in H_2O solution according to the procedure
of Robins, M.J., et al., J Med Chem (1984) 27:1486-1492
until the bromine color persisted (3-4 ml). After
stirring 30 minutes at room temperature, the color was
discharged with crystals of $NaHSO_3$. The reaction was
filtered, then washed with H_2O and EtOH, to collect 99
mg (87%) white solid which appeared to be homogenous by
thin layer chromatography using SiGF: R_f=0.33 using
CH_3CN:0.1N NH_4Cl(7:3). 'H NMR (DMSO-d_6):δ 1.19 (tr,
3H), 1.3-1.9 (m, 4H), 3.53 (tr, 2H), 3.92 (dq, 2H), 5.33
(S, 2H), 6.68 (br s, 2H). UV λmax (ε): pH 1, 260
(16,400); pH 7, 260 (16,400), pH 11, 269 (13,600).

HPLC examination of the compound on a Vydac
TP-21854 column (4.6 x 250 mm) with a 15 min non-linear
gradient of 0 to 100% 'B' in 'A', where 'A' is 0.0125N
$Et_3N\cdot HCO_2H$, pH 3.0 and B is 5% aqueous CH_3CN (program #8

-30-

on Waters Model 660 solvent programmer) indicated about
5% starting material. Chromatography on DEAE sephadex
as related in Example 1 using a linear gradient of water
and 0.1N NH_4 HCO_3 yielded product title compound free of
5   starting material.

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-31-

Example 10
Preparation of 9(3'-Ethylphosphono-3'-Hydroxy-1'-propyloxymethyl)guanine

1. EtOH
2. CH$_2$=CHCHO

$$(EtO)_2PCl \longrightarrow CH_2=CHCHP(OEt)_2 \longrightarrow CH_2=CHCHP(OEt)_2$$

with OH on the first product and OSi(CH$_3$)$_2$—tBu on the second product.

$$HOCH_2-CH_2CHP(OEt)_2$$
OSi(CH$_3$)$_2$
tBu

CH$_2$O,
HCl

$$ClCH_2OCH_2CH_2CHP(OEt)_2$$
OSiMe$_2$
tBu

silated
6-chloroguanine (6-Cl-G)

$$6-Cl-G-CH_2OCH_2CH_2CHP(OEt)_2$$
OSi
tBu

$$G-CH_2OCH_2CH_2CH \quad POEt$$
OH OH

A. Preparation of Diethyl 1-t-butyldimethylsilyloxy allyl phosphonate.

A solution of 68.8 g (0.355 mole) diethyl 1-hydroxyallyl phosphonate, prepared as reported by

Gazizov et al, <u>J Gen Chem USSR</u> (1977) <u>47</u>:2465, (Eng.
Trans., p. 2253), in 120 ml dry DMF was combined with
60.44 g (0.888 mole) imidazole and treated with 63.87 g
(0.424 mole) t-butyldimethylchlorosilane according to
the procedure of Corey, E.J., et al, in <u>J Am Chem Soc</u>
(1972) <u>94</u>:6190. The reaction was stirred at room
temperature for 24 hours and combined with 750 ml Et$_2$O.
The biphasic solution was washed with H$_2$O (3 x 250 ml).
The organic layer was dried with MgSO$_4$, gravity
filtered, and evaporated to dryness for a quantitative
yield (110 g) of a colorless oil. Thin-layer
chromatography showed a spot on SiGF (10% EtOAc in CHCl$_3$
visualization with 2% phosphomolybdic acid plus heat) at
Rf 0.44 with trace impurity at Rf 0.61. ^{1}H NMR (CDCl$_3$):
δ 0.10 and 0.13 (s, s, 5H), 0.97 (s, 9H), 1.33 (tr, 6H),
4.20 (dq, 4H), 4.57 (dd, 1H), 5.10-5.63 (m, 2H),
5.87-6.34 (m, 1H).

B. <u>Preparation of Diethyl 1-t-butyldimethylsilyloxy-3-
hydroxypropyl phosphate.</u>

Diethyl 1-t-butyldimethylsilyloxyallyl
phosphonate (76 g, 0.247 mole) was azeotroped from
toluene three times and dissolved in 400 ml dry THF. 1
M diborane in THF (150 ml) was added dropwise at 25°C
over 45 minutes and the reaction then stirred an
additional 1 hour. The hydroboration was terminated by
addition of 65 ml H$_2$O maintaining the temperature at
20°C with ice cooling. 3 N NaOH (20 ml) was added
followed by dropwise addition of 80 ml 30% H$_2$O$_2$ with ice
cooling to keep temperature at 40°C. The pH was checked
intermittently and, when no longer alkaline, another 20
ml plus 10 ml 3 N NaOH was added in 2 portions. After
addition of H$_2$O$_2$, the reaction was maintained at 35°C
for an additional 20 minutes. EtOH (40 ml) was added to

improve miscibility. THe reaction was poured into 800
ml Et_2O; 300 ml H_2O plus 25 ml solid NaCl was added,
stirred well, and placed in the refrigerator overnight.
The layers were separated and the Et_2O layer was washed
thoroughly with H_2O saturated with NaCl to minimize
emulsions. The Et_2O solution was dried with $MgSO_4$,
filtered, and evaporated to yield 70.25 g (87%) yellow
oil. Thin-layer chromatography on SiGF (EtOAc, 5%
phosphomolybdic acid plus heat) showed two spots at Rf
0.35 and 0.55 in approximately 2:3 ratio. In a previous
synthesis, the material at Rf 0.55 was identified as the
secondary alcohol and the material at Rf 0.35 as the
primary alcohol. ^{1}H NMR ($CDCl_3$) δ 0.083 and 0.113 (s,
s, 3H, 3H, $-Si(CH_3)_2$), 0.882 (s, 9H, $SiC(CH_3)_3$), 1.314
and 1.317 (tr, tr, 6H, $-P(OCH_2C\underline{H}_3)_2$), 1.9-2.1 (m, 2H,
$HOCH_2C\underline{H}_2-$), 3.69 and 3.87 (ddq, dq, 2H, $HOC\underline{H}_2-$),
4.07-4.22 (m, 5H, $-POC\underline{H}_2CH_3$ and $-C\underline{H}PO-$). In order to
enhance chromatographic separation of the two alcohols,
the crude material was tritylated according to the
procedure of Chaudhary et al, Tet. Letters (1979) 95.
The mixture of tritylated alcohols were separated on
silica eluting with increments of CH_2Cl_2, 10%, 30%, and
50% EtOAc in CH_2Cl_2, and finally EtOAc. TLC on SiGF
(10% EtOAc in CH_2Cl_2) of the tritylated primary alcohol
showed R_f 0.6. The trityl group was removed
instantaneously at 0°C with 1% HCl in $CHCl_3$ according to
the procedure of Choy and Unrau, Carbohydrate Res.
(1971) 17:439. Silica chromatography eluting with
CH_2Cl_2, 10% EtOAc in CH_2Cl_2, and EtOAc provided 6.6 g of
diethyl 1-t-butyldimethyl-silyloxy-3-hydroxypropyl
phosphonate.

-34-

C.   Preparation of 6-Chloro-9(3'-diethyl phosphono-3'-t-
butyldimethylsilyloxy-1'-propyloxymethyl)guanine.

The coupling of (0.020 mole) diethyl 1-t-butyl
dimethylsilyloxy-3-chloromethoxypropyl phosphonate and
5   6-chloroguanine was conducted according to the procedure
set forth for 6-chloro-9(3'-diethyl phosphono-1'
-propyloxy methyl)guanine in Example 1. Silica
chromatography eluting with 10:1 EtOAc:iPrOH afforded
2.5 g (26%) white solid, Rf 0.79, mp 120-123°C. ^{1}H NMR
10   (CDCl$_3$) δ 0.03 and 0.14 (s, s, 6H), 0.91 (s, 9H), 1.35
and 1.38 (tr, tr, 6H), 1.77-2.32 (m, 2H), 3.64 (tr, 2H),
3.88-4.27 (m, 5H), 5.24 (br s, 2H), 5.45 (s, 2H), 7.94
(s, 1H).

15   D.   Preparation of 6-Chloro-9(3'-diethylphosphono-3'-
hydroxy-1'-propyloxymethyl)guanine.

The t-butyldimethylsilyl group was removed
from 1.62 g (3.4 mmol)
6-chloro-9(3-diethylphosphono-3'-t-butyldimethylsilyloxy
20   -1'-propyloxymethyl)guanine in 30 ml dry THF by addition
of 3.75 ml 1 M tetrabutylammonium fluoride at 5°C.
After stirring 22 minutes at 5°C, the reaction was
evaporated to dryness and chromatographed in silica
eluting with 1:1 EtOAc:iPrOH. 6-Chloro-9(3'-diethyl-
25   phosphono-3'-hydroxy-1'-propyloxymethyl)guanine was
obtained by evaporation of the appropriate fractions and
crystallization from EtOAc to yield 0.95 g (71%) white
solid, mp 116-118°C. Thin-layer chromatography on SiGF
(10:1 EtOAc · iPrOH) showed Rf 0.21. Microanalysis
30   confirmed the formula (C$_{13}$H$_{21}$ClN$_5$O$_5$P). Calc: C-39.65%,
H-5.38%, N-17.79%. Found: C-39.59%, H-5.54%, N-17.32%.
^{1}H NMR (CDCl$_3$): δ 1.31 (tr), 1.82-2.15 (m), 3.65-3.85
(m), 3.92-4.42 (ml, 5.46 (s), 5.59 (brs), 7.85 (s). UV
λ$_{max}$: 245, 306.

-35-

E. <u>Conversion to Monoester and Dehalogenation</u>

The hydrolysis of 0.645 g (1.64 mmol) of 6-chloro-9(3'-diethyl phosphono-3'-hydroxy-1'
-propyloxymethyl)guanine with 32.5 ml 1 N NaOH was conducted as set forth in Example 3 for the synthesis of 9(3'-ethyl phosphono-1'-propyloxymethyl)guanine. DEAE-Sephadex chromatography yielded 35 mg (6%) material with R_f 0.48 on SiGF (7:3 CH_3CN: 0.1 N NH_4Cl). ^1H NMR (D_2O): δ 1.31 (tr), 1.72-2.20 (m), 3.76-4.1 (m), 5.68 (s). UV λ_{max}: pH 1 256, 278 shoulder; pH 7 251, 270 shoulder; pH 11 255, 265 shoulder. Mass spectrum m/e 635 (M^+ for TMS_1 derivative).

<u>Example 11</u>

Using the procedures described above, the following compounds of the invention were prepared.

-36-

Table 1

Compound #	B	R1	R2	n	Form
1	G	H	H	1	phosphonic acid
2	G	H	H	1	monoethylester
3	G	H	H	1	diethyl ester
4	G	H	H	1	monoethyl ester, monosodium salt
5	G	CH2OH	H	1	monoethyl ester
6	G	CH2OH	H	1	phosphonic acid
7	G	CH3	H	1	monoethyl ester
8	G	H	H	5	monoethyl ester
9	G	CH2OH cyclic structure 1a	H	1	phosphonic acid
10	*	H	H	1	diethyl ester
11	*	H	H	5	diethyl ester
12	**	H	H	1	monoethyl ester
13	***	H	H	1	monoethyl ester
14	G	H	OH	1	monoethyl ester
15	*	CH2OH	H	1	diethyl ester (acetoxy ester)
16	*	H	OH	1	diethyl ester

* 6-chloroguanine(6-chloro-2-aminopurine).
** 8-bromoguanine.
*** 2-NH2-adenine (2,6-diaminopurine).

F. Biological Testing

The compounds of the invention were evaluated
in vitro as antiviral agents against herpes virus and

-37-

against RNA retrovirus. Various herpes strains were
used. An infected B lymphoblastoid cell line was used
as HIV substrate to test antiviral activity with respect
to RNA retrovirus.

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Activity Against Herpes-Type Virus

        One herpes virus strain employed was Strain
McCrae of type 1 herpes (thymidine kinase positive
virus) (HSV-1TK$^+$). This strain was prepared and titered
10   in MA-104 cells and frozen at -90°C until use. Also
used were strain HF (HSV-1TK$^-$), strain E194 (HSV-2), NJB
strain (MCMV), strain AD169 (HCMV).
        Continuous passaged monkey kidney (MA-104)
cells were used for testing of herpes-type virus, with
15   growth medium consisting of Minimum Essential Medium
(MEM) supplemented with 0.1% NaHCO$_3$ and 50 µl
gentamicin.
        To a 96 well microtiter plate containing an
established 24 hour monolayer of cells from which the
20   medium has been decanted was added 0.1 ml of varying
(one-half log$_{10}$) concentrations of test compound, which
incubated on the cell 15 minutes, after which 0.1 ml of
virus in a concentration of 320 cell culture 50%
infectious doses (CCID$_{50}$)/0.1 ml was added. The plate
25   was covered with plastic wrap and incubated at 37°C.
Included with the test were toxicity controls (each
concentration of compound + test medium in place of
virus), virus controls (virus + test medium in place of
compound) and cell controls (test medium in place of
30   compound and virus). The cells were examined
microscopically after 72 hours for evidence of
cytotoxicity and for viral cytopathic effect (CPE).
Vidarabine was run on the same plate in parallel. The
test compounds were added to the medium at a

-38-

concentration of 2000 µg/ml for use as a positive
control.

Antiviral activity was determined by
observation of inhibition of viral CPE. This activity
was expressed by ED_{50}, defined as that dose range of
compound causing 50% CPE inhibition.

A Virus Rating (VR) was also determined. VR
is a numerical expression of antiviral activity,
weighted to take into account any cytotoxicity observed,
as discussed by Sidwell et al, Appl Microbiol (1971)
22:797. Generally, a VR of 0.1-0.4 indicates slight
antiviral effect, 0.5-0.9 indicates moderate antiviral
effect, and $\frac{1}{2}$ 1.0 indicates strong antiviral effect.

The results of the foregoing assays are as
follows: The activities of compounds 1 and 2,
(9-(3'-phosphono-1'-propoxymethyl)guanine and its
monoethyl ester), against HSV-1TK^{+} were comparable to
that of vidarabine, as shown in Tables 2 and 3.

Table 2

Anti HSV-1TK^{+} Activity

Compound 1		Vidarabine	
Conc. (ug/ml)	CPE Inhib. (%)	Conc. (ug/ml)	CPE Inhib. (%)
1000	100	1000	100
320	94	320	100
100	79	100	87
32	62	32	87
10	49	10	69
3.2	28	3.2	28
1.0	31	1.0	56
VR	1.4	1.3	
ED_{50}	10 µg/ml	10 µg/ml	

-39-

An additional test of Compound 1 gave VR against
HSV-1TK[+] of 0.6; this compound was not active against
murine CMV.

5

Table 3

Anti HSV-ITK[+] Activity

	Compound 2			Vidarabine	
Conc. (µg/ml)	Test 1 CPE Inhib. (%)	Test 2 CPE Inhib. (%)	Conc. (µg/ml)	Test 1 CPE Inhib. (%)	Test 2 CPE Inhib. (%)
1000	100	76	1000	100	100
320	82	67	320	100	100
100	47	57	100	96	85
32	6	57	32	96	57
10	38	48	10	60	39
3.2	96	52	3.2	2	0
1.2	69	48	1.0	0	0
1000	100	76	1000	100	100
320	82	67	320	100	100
100	47	57	100	96	85
32	6	57	32	96	57
10	38	48	10	60	39
3.2	96	52	3.2	2	0
1.2	69	48	1.0	0	0
VR	>2.0	>1.4		0.8	0.7
ED50	<1.0µg/ml	<1.0µg/ml		10µg/ml	10µg/ml
MTD*	320	>1000		10	10

*Maximum tolerated dose, µg/ml.

Additional tests of Compound 2 against HSV-1TK^{+} showed
VR values of 0.4, 0.6, 0.9, 0.4, 0.3, and 0.7. Compound
2 was active against murine CMV (VR = 0.6, 1.6, 2.3) and
human CMV (ED50 = 10 µg/ml).

Compound 5 (9-(3'-phosphono-1'-hydroxy-
methylpropoxymethyl) guanine, monoethyl ester, showed an

-40-

ED$_{50}$ of 0.1-3.2 µg/ml against human CMV. It was mildly
effective against HSV1TK$^+$ and HSV-2.

Compound 2 also was tested _in vivo_ in guinea
pigs as an agent against HSV-1TK$^+$. The animals were
inoculated with the virus; 18 hours later Compound 2 at
two concentrations (0.4% and 1-2% (saturated) solution
in water) was administered, and five days later blister
diameters at the point of inoculation were measured. A
5% solution of acyclovir or a 1.4% solution of
poly(vinylalcohol) was used as control. Satellite
lesions were measured, as well.

The results of these tests are given as
average number of lesions in Table 4 and show that
Compound 2 has activity against HSV TK$^+$ virus, at least
in saturated solution.

Table 4

Virus	Placebo poly(Vinylalcohol)	Acyclovir	Compound 2 1-2%	Compound 2 0.4%
TK$^+$	1.7	1.0	0.9	2.1
Satellite lesions	9	4	6	11

Table 5 shows the results of two experiments
showing the antiviral activity of compounds 2 and 5
against human cytomegalovirus.

-41-

Table 5

Compound Concentration (µg/ml)	Compound 2		Compound 5		Acyclovir	
	No. Plaques	% Reduct.	No. Plaques	% Reduct.	No. Plaques	% Reduct.
Experiment No. 1						
100	0	100	1	94	1	94
32	5	71	2	88	7	59
0	17	-	17	-	17	-
Approx. ED50[a] (µg/ml):	10		0.1		10-32	
Experiment No. 2						
100	1	96	0	100	1	96
32	6	73	3	86	8	64
10	17	23	7	68	20	9
0	22	-	22	-	22	-
Approx. ED50 (µg/ml):	10-32		3.2		10-32	
Approx. MTD[b]:	1000		1000		1000	

[a]50% effective dose, determined from plot of data.

[b]Maximum tolerated dose: That dose causing approximately 50%
cytotoxic effects in cells, determined by the microscopic
examination of cells for sloughing, shape alteration, and
granularity in separately run control tests in 96-well microplates.

        The data on acyclovir were gathered from tests
run in MRC-5 cells grown under agarose overlay, with
plaques read 7 days after virus exposure. Overall, the
data in Table 5 show that compound 1 and acyclovir
appear to have approximately the same human
CMV-inhibitory effects and cytotoxicity. Compound 5
appears to have a more potent anti-CMV effect than
acyclovir, and to be no more cytotoxic.

-42-

Activity Against RNA Retrovirus

 The ability of compounds of the invention to
exhibit antiviral activity against RNA retroviruses was
shown in an in vitro assay similar to that described
5 above and was also demonstrated by their ability to
inhibit reverse transcriptase.

 For the reverse transcriptase assay, cloned
reverse transcriptase (RT) (Bethesda Research
Laboratories) from Moloney murine leukemia virus (MMLV)
10 or avian myeloblastosis virus (AMV) (Seikagaku or
Boehringer Mannheim) was used to direct DNA synthesis
from a polyribonucleotide template and a
oligodeoxynucleotide primer under reaction conditions
(Houts, G.E., et al, J Virol (1979) 29:517.)
15 Reaction mixtures (100 μl) for the assay of
MMLV RT consisted of 50 mM Tris-hydrochloride, pH 8.0, 6
mM MgCl$_2$, 40 mM KCl, 100 μg bovine serum albumin per ml,
1 mM dithiothreitol, 0.1 mM polyadenylic acid
(Pharmacia, Inc.), 01 mM oligo(dT)$_{12-18}$ (Pharmacia,
20 Inc.), 0.4 mM deoxythymidine triphosphate (dTTP), and
0.1 mM tritiated (93.5 Cl/mmol) dTTP (New England
Nuclear). The compounds assayed were added in various
concentrations and dilutions at a volume of 10 μl.
Results were confirmed by duplicate assays. Dilutions
25 were made using 10% aqueous DMSO. The total activity
was measured by spotting on dry Whatman 3 mm filter
disks and radioactivity was determined by liquid
scintillation techniques. The assay time course was
begun by adding 10 units of RT from MMLV or AMV and
30 incubating the reaction at 37°C. Aliquots (10 μl) were
removed as a function of time and quenched in 20 μl of
stop mix [0.25 mM EDTA, 0.5 mg/ml yeast tRNA (BDH
Biochemicals, England), 10 mM sodium pyrophosphate]. The
30 μl samples were spotted onto Whatman 3 mm disks and

-43-

batch-washed (10 ml/disk) in ice-cold 10% TCA, 1% sodium
pyrophosphate for 10 minutes with agitation. This was
followed by three 4 minute ice-cold 5% TCA batch washes
and a final 95% ethanol rinse (1 minute). Disks were
5   dried under a heat lamp and the acid-insoluble product
radioactivity was determined by liquid scintillation
counting. Blank assays were conducted using 10% DMSO.
Phosphonoformic acid, trisodium salt (Sigma Chemical
Company), dissolved in water was used as a positive
10   control at a final concentration of 1 mM.

        To test antiviral activity versus HIV, the
following procedure was used: Infectious HIV was
assayed in cell culture as described originally by
Barre-Sinoussi et al for isolation of LAV, except that
15   persistently infected lymphoblastoid cells were used
instead of mononuclear cells from anti-LAV negative
donors. HIV obtained from cell culture was stored in
liquid nitrogen in aliquots of RPMI 1640 culture medium
with 20% of total calf serum and 20% glycerol and
20   containing 1,000 TCID-50 HIV. The lymphoblastoid cells
(10^6) were incubated with 1,000 TCID-50 of HIV obtained
from cell cultures, and cells were then cultured in
RPMI-1640 medium. Culture medium was changed every 3
days for 3 weeks and the medium for each 3-day period
25   from Day 6 through Day 21 was assayed for viral reverse
transcriptase activity by concentrating (pelleting)
virus by ultracentrifugation in the 100 place Spinco 25
rotor and assaying pellets for reverse transcriptase
(RT) using a ^{32}P-dTTP in the presence of dimer-template
30   poly rA-oligo dT. Compounds to be tested for
neutralization of infection were incubated with HIV
aliquots (10^3 TCID-50) for 30 minutes at 22°C before
incubation with 10^6 lymphoblastoid cells.

-44-

The results obtained in the foregoing assays
were as follows: Compound 1, 9-(3-phosphono-1-propoxy
methyl)guanine, showed 75% inhibition of reverse
transcriptase at 8 mM concentration; compound 2, the
5   monoethyl ester form, showed 100% inhibition at 1 mM.
These activities were further reflected in ED_{50} values
against HIV, which were 40-50 µg/ml and 30µg/ml,
respectively.

10   G.   Formulations
The following formulations based on the
compounds of the invention and their preparation are
representative.
A formulation suitable for injections
15   intramuscularly or intraperitoneally is prepared by
combining the first four of the following materials:

Compound of the invention	1 g
Poly(ethylene glycol)	50 g
Propylene glycol	50 g
Tween-80 suspension agent	1.5 g
Injectable saline	200 ml

and then adding the last material. The material forms a
25   clear solution which is filtered and sealed in sterile
containers.
A simple intravenous injection formulation is
formed by dissolving 1 gram of an active compound in 250
ml of injectable saline which after filtering is
30   packaged in sterile bottles.
A cream for topical administration is
formulated by stirring 10 g of active compound of the
invention with 20 g of mineral oil, 40 g of petroleum
jelly, 0.3 g of mixed methyl/propyl paraben, and 5 g of

-45-

nonionic surfactant at 50°C. Then 150 ml of water are
stirred into the mixture at 50°C at high speed to form a
cream, and the mixture is cooled and packaged in capped
tubes.

5   An oral dosage form is prepared from 10 g of a
compound of the invention, 100 g of lactose, and 1 g of
starch, which are mixed with 0.1 g of magnesium stearate
in methanol to granulate. The methanol is removed by
gentle heating with stirring. A portion of this
10   material is retained as a granular powder for oral use
while the remainder is hand formed into 250 mg tablets
in a manual tableting machine.

              The foregoing examples and formulations have
15   been presented to illustrate the present invention and
are not to be construed as limitations on the
invention's scope, which is instead defined by the
following claims.

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-46-

WHAT IS CLAIMED IS:

    1. A compound of the formula

$$B-CH_2-O-\underset{\underset{R1}{|}}{C}H(CH_2)_n\underset{\underset{R2}{|}}{C}H\overset{\overset{O}{\|}}{P}(OH)_2 \qquad (1)$$
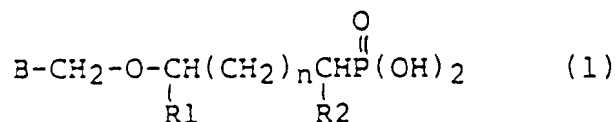
    wherein B is a substituted or unsubstituted purine base conjugated to the remainder of the structure through the 9-position,
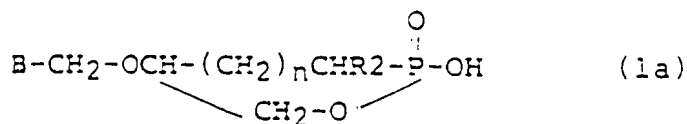
    R1 is selected from H, methyl, hydroxymethyl and its lower alkyl esters, halomethyl, azidomethyl and cyano;

    R2 is selected from H, methyl, hydroxymethyl and its lower alkyl esters, halomethyl, azidomethyl, cyano and OH and its lower alkyl esters; or R2 and the H residing in the same carbon are, together, =O; and

    n is an integer of 0-5,

    and the pharmaceutically acceptable acid addition salts, mono- and dibasic salts, and mono- and diesters thereof,

    or wherein, when n is 0 or 1 and R1 is CH₂OH, the compound of Formula 1 has the formula

$$B-CH_2-O\underset{\diagdown CH_2-O\diagup}{CH-(CH_2)_nCHR2-}\overset{\overset{O}{\|}}{P}-OH \qquad (1a)$$

    wherein B and R2 are as above defined,

    and the pharmaceutically acceptable acid addition salts, monobasic salts, and monoesters thereof.

-47-

2. The compound of claim 1 wherein B is
selected from guanine, 8-haloguanine, adenine,
8-haloadenine, 2-hydroxy-6-halopurine, and
2-amino-6-halopurine (6-haloguanine), 2-aminopurine and
5   2,6-diaminopurine.

3. The compound of claim 1 wherein the
phosphonic acid ester or diester is the alkyl (1-8C)
ester or diester.
10

4. The compound of claim 3 which is selected
from the free phosphonic acid, the phosphonic acid
monoethyl ester, and the phosphonic acid diethyl ester.

15   5. The compound of claim 1 wherein R1 is
selected from H, CH_3, and CH_2OH, and R2 is H or OH.

6. The compound of claim 1 wherein n is 1 or
2.
20

7. The compound of claim 1 which has the
formula
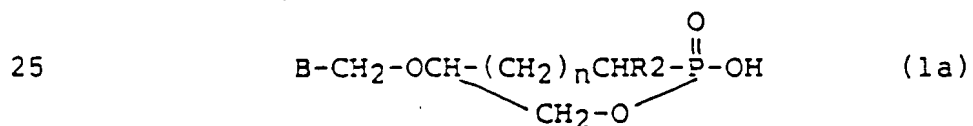
25          $$B\text{-}CH_2\text{-}OCH\text{-}(CH_2)_nCHR2\text{-}\overset{\overset{O}{\|}}{P}\text{-}OH \qquad (1a)$$
$$\diagdown CH_2\text{-}O \diagup$$

and the pharmaceutically acceptable acid
30   addition salts, monobasic salts and mono esters thereof.

8. The compound of claim 6 wherein B is
guanine or 6-haloguanine.

-48-

9.   The compound of claim 1 wherein B is guanine, R1 and R2 are H, and n is 1 as the phosphonic acid, monoethyl ester, diethyl ester, or monoethyl ester/monosodium salt

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10.   The compound of claim 1 wherein B is guanine, R1 and R2 are H, and n is 5 as the phosphonic acid, monoethyl ester, diethyl ester, or monoethyl ester/monosodium salt

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11.   The compound of claim 4 which is selected from the group consisting of:

6-chloro-9(3'-diethylphosphono-1'-propyloxymethyl)
15   guanine;
9(3'-diethylphosphono-1'-propyloxymethyl) guanine;
9(3'-ethylphosphono-1'-propyloxymethyl)guanine;
9(3'-phosphono-1'-propyloxymethyl)guanine;
9(3'-ethylphosphono-1'-propyloxymethyl)guanine,
20   monosodium salt;
6-chloro-9(7'-diethylphosphono-1'-heptyloxymethyl)
       guanine;
9(7'-ethylphosphono-1'-heptyloxymethyl)guanine;
9(3'-ethylphosphono-1'-hydroxymethyl-1'-propyloxymethyl)
25   guanine;
6-chloro-9(3'-diethylphosphono-1'-acetoxymethyl-1'-
       propyloxymethyl)guanine;
9(3'-phosphono-1'-hydroxymethyl-1'-propyloxymethyl)
       guanine;
30   9(3'-ethylphosphono-1'-methyl-1'-propyloxymethyl)
       guanine;
9(3'-phosphono-1'-hydroxymethyl-1'-propyloxymethyl)
       guanine, cyclic ester;

-49-

9(3'-ethylphosphono-3'-hydroxy-1'-propyloxymethyl)
   guanine;

6-chloro-9(3'-diethylphosphono-3'-hydroxy-1'-
   propyloxymethyl)guanine;

5   8-bromo-9(3'-ethylphosphono-1'-propyloxymethyl)guanine;
     and

2-amino-9(3'-ethylphosphono-1'-propyloxymethyl)adenine.

          12.   A pharmaceutical composition for treating
10   herpes viral infection which comprises an effective
      amount of the compound of claim 1 in admixture with a
      suitable pharmaceutically acceptable excipient.

          13.   A method to treat herpes infection in an
15   infected subject which comprises administering to said.
      subject an effective amount of the compound of formula 1
      or pharmaceutical compositions thereof.

          14.   A pharmaceutical composition for treating
20   an RNA retroviral infection which comprises an effective
      amount of the compound of claim 1 in admixture with a
      suitable pharmaceutically acceptable excipient.

          15.   A method to treat an RNA retroviral
25   infection in an infected subject which comprises
      administering to said subject an effective amount of the
      compound of formula 1 or pharmaceutical compositions
      thereof.

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1. A compound of the formula

$$B-CH_2-O-\underset{R1}{CH}(CH_2)_n\underset{R2}{CH}\overset{O}{P}(OH)_2 \qquad (1)$$
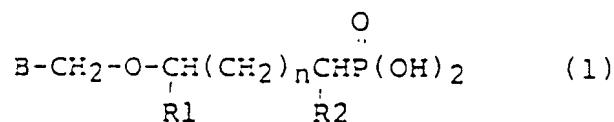
wherein B is a purine base conjugated to the
remainder of the structure through the 9-position,
R1 is selected from H, methyl, hydroxymethyl
and its lower alkyl esters, halomethyl, azidomethyl and
cyano;
R2 is selected from H, methyl, hydroxymethyl
and its lower alkyl esters, halomethyl, azidomethyl,
cyano and OH and its lower alkyl esters; or R2 and the H
residing in the same carbon are, together, =O; and
n is an integer of 0-5,
and the pharmaceutically acceptable acid addi-
tion salts, mono- and dibasic salts, and mono- and
diesters thereof,
or wherein, when n is 0 or 1 and R1 is CH2OH,
the compound of Formula 1 has the formula

$$B-CH_2-OCH-(CH_2)_nCHR2-\overset{O}{P}-OH \qquad (1a)$$
$$\diagdown CH_2-O\diagup$$

wherein B and R2 are as above defined,
and the pharmaceutically acceptable acid addi-
tion salts, monobasic salts, and monoesters thereof.

9. The compound of claim 1 wherein B is
guanine, R1 and R2 are H, and n is 1 as the phosphonic
acid, monoethyl ester, diethyl ester, or monoethyl
ester/monosodium salt

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10. The compound of claim 1 wherein B is
guanine, R1 and R2 are H, and n is 5 as the phosphonic
acid, monoethyl ester, diethyl ester, or monoethyl
ester/monosodium salt

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[Claim 11 canceled.]

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[Claim 11 canceled (continued).]

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12.   A pharmaceutical composition for treating
herpes viral infection which comprises an effective
amount of the compound of claim 1 in admixture with a
10   suitable pharmaceutically acceptable excipient.

13.   A method to treat herpes infection in an
infected subject which comprises administering to said
subject an effective amount of the compound of formula 1
15   or pharmaceutical compositions thereof.

14.   A pharmaceutical composition for treating
an RNA retroviral infection which comprises an effective
amount of the compound of claim 1 in admixture with a
20   suitable pharmaceutically acceptable excipient.

15.   A method to treat an RNA retroviral
infection in an infected subject which comprises admin-
istering to said subject an effective amount of the com-
25   pound of formula 1 or pharmaceutical compositions
thereof.

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16. (New claim)　The compound of claim 4 which
is selected from the group consisting of:

2-amino-6-chloro-9-(3'-diethylphosphono-1'-
propyloxymethyl) purine;

9(3'-diethylphosphono-1'-propyloxymethyl)
guanine;

9(3'-ethylphosphono-1'-propyloxymethyl)
guanine;

9(3'-ethylphosphono-1'-propyloxymethyl)
guanine, monosodium salt;]

2-amino-6-chloro-9(3'-diethylphosphono-1'-
heptyl-oxymethyl) purine;

9(3'-ethylphosphono-1'-heptyloxymethyl)
guanine;

2-amino-6-chloro-9(3'-diethylphosphono-1'-
acetoxy-methyl-1'-propyloxymethyl) purine;

9(3'-phosphono-1'-hydroxymethyl-1'-propyloxy-
methyl) guanine;

9(3'-ethylphosphono-1'-methyl-1'-propyloxy-
methyl) guanine;

9(3'-ethylphosphono-3'-hydroxy-1'-propyloxy-
methyl) guanine;

2-amino-6-chloro-9(3'-diethylphosphono-3'-
hydroxy-1'-propyloxymethyl) purine;

8-bromo-9(3'-ethylphosphono-1'-propyloxy-
methyl) guanine; and

2-amino-9(3'-ethylphosphono-1'-propyloxy-
methyl) adenine.

17. (New claim) The compound of claim 1, given
by formula (1), wherein R1 is H and either R2 is H or R2
and the H residing in the same carbon are, together =O,
and the pharmaceutically acceptable acid addition salts,
monobasic salts, and monoesters thereof.

18. (New claim) The phosphonic acid mono ester
of the compound of claim 1, wherein the ester is 1-8C
alkyl, phenyl or benzyl, optionally substituted with one
5   or two halo, hydroxy, or alkoxy residues.

19. (New claim) The phosphonic acid mono ester
of the compound of claim 7, wherein the ester is 1-8C
alkyl, phenyl or benzyl, optionally substituted with one
10   or two halo, hydroxy, or alkoxy residues.

20. (New claim) The phosphonic acid mono ester
of the compound of claim 17, wherein the ester is 1-8C
alkyl, phenyl or benzyl, optionally substituted with one
15   or two halo, hydroxy, or alkoxy residues.

21. (New claim) The phosphonic acid mono ester
of the compound of claim 18, wherein the ester is 1-6C
alkyl, optionally substituted with one or two halo,
20   hydroxy, or alkoxy residues.

22. (New claim) The phosphonic acid mono ester
of the compound of claim 19, wherein the ester is 1-6C
alkyl, optionally substituted with one or two halo,
25   hydroxy, or alkoxy residues.

23. (New claim) The phosphonic acid mono ester
of the compound of claim 20, wherein the ester is 1-6C
alkyl, optionally substituted with one or two halo,
30   hydroxy, or alkoxy residues.

24. (New claim) The phosphonic acid of claim
7.

25. (New claim) The compound of claim 1 which is selected from the group consisting of 9(3'-phosphono-1'-propyloxymethyl) guanine, 9(3'-ethylphosphono-1'-propyloxymethyl) guanine monosodium salt, 9(3'-ethylphosphono-1'-propyloxymethyl) guanine ammonium salt, 9(3'-ethyl-phosphono-1'-hydroxymethyl-1'-propyloxymethyl) guanine, and 9(1',3'-phosphono-1'-hydroxymethyl-1'-propyl-oxymethyl) guanine cyclic ester.

26. (New claim) The compound of claim 1 which is 9(3'-phosphono-1'-propyloxymethyl) guanine.

27. (New claim) The compound of claim 1 which is 9(3'-ethylphosphono-1'-propyloxymethyl) guanine, monosodium salt.

28. (New claim) The compound of claim 1 which is 9(3'-ethylphosphono-1'-hydroxymethyl-1'-propyloxymethyl) guanine.

29. (New claim) The compound of claim 1 which is 9(1',3'-phosphono-1'-hydroxymethyl-1'-propyloxymethyl) guanine, cyclic ester.

30. (New claim) The compound of claim 1 which is 9(3'-methylphosphono-1'-propyloxymethyl) guanine.

31. (New claim) The compound of claim 1 which is 9(3'-butylphosphono-1'-propyloxymethyl) guanine.

32. (New claim) The compound of claim 1 which is 9(3'-benzylphosphono-1'-propyloxymethyl) guanine.

33. (New claim) The compound of claim 1 which
is 9(3'-phenylphosphono-1'-propyloxymethyl) guanine.

34. (New claim) The compound of claim 1 which
is 9[3'-(p-methoxyphenylphosphono)-1'-propyloxymethyl]
guanine.

35. (New claim) The compound of claim 1 which
is 9(3'-ethylphosphono-3'-hydroxy-1'-propyloxymethyl)
guanine.

36. (New claim) The compound of claim 1 which
is given by the structure
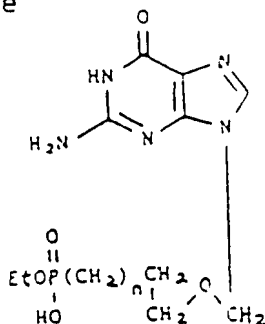
where n is 0, 2, 3, 4, or 5.

37. (New claim) The compound of claim 36 which
is 9(2'-ethylphosphono-1'-ethyloxymethyl) guanine.

38. (New claim) The compound of claim 36 which
is 9(4'-ethylphosphono-1'-butyloxymethyl) guanine.

39. (New claim) The compound of claim 36 which
is 9(5'-ethylphosphono-1'-pentyloxymethyl) guanine.

40. (New claim) The compound of claim 36 which
is 9(6'-ethylphosphono-1'-hexyloxymethyl) guanine.

41. (New claim) The compound of claim 36 which is 9(7'-ethylphosphono-1'-heptyloxymethyl) guanine.

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42. (New claim) A pharmaceutical composition for treating herpes viral infection which comprises an effective amount of the compound of claim 7.

43. (New claim) A method to treat herpes

10 infection in an infected subject which comprises administering to said subject an effective amount of the compound of claim 7 or pharmaceutical compositions thereof.

44. (New claim) A pharmaceutical composition

15 for treating an RNA retrovirus which comprises an effective amount of the compound of claim 7.

45. (New claim) A method to treat an RNA retrovirus infection in an infected subject which comprises administering to said subject an effective amount

20 of the compound of claim 7 or pharmaceutical compositions thereof.

46. (New claim) The pharmaceutical composition

25 of claim 44, wherein the RNA retrovirus is HIV.

47. (New claim) The method of claim 45, wherein the RNA retrovirus is HIV.

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48. (New claim) A pharmaceutical composition for treating herpes viral infection which comprises an effective amount of the compound of claim 17.

49. (New claim) A method to treat herpes infection in an infected subject which comprises administering to said subject an effective amount of the compound of claim 17 or pharmaceutical compositions thereof.

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50. (New claim) A pharmaceutical composition for treating an RNA retrovirus infection which comprises an effective amount of the compound of claim 17.

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51. (New claim) A method to treat an RNA retrovirus infection in an infected subject which comprises administering to said subject an effective amount of the compound of claim 17 or pharmaceutical compositions thereof.

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52. (New claim) The pharmaceutical composition of claim 50, wherein the RNA retorvirus is HIV.

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53. (New claim) The method of claim 51, wherein the RNA retrovirus is HIV.

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INTERNATIONAL SEARCH REPORT

International Application No PCT/US 87/03446

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC^4: C 07 F 9/65; A 61 K 31/675

II. FIELDS SEARCHED

Minimum Documentation Searched

Classification System	Classification Symbols
IPC^4	C 07 F 9/00

Documentation Searched other than Minimum Documentation
to the Extent that such Documents are Included In the Fields Searched

III. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of Document, with Indication, where appropriate, of the relevant passages	Relevant to Claim No.
X	Antiviral Research, volume 6, 1986, Elsevier Science Publishers B.V. (Biomedical Division), A.E. Duke et al.: "In vitro and in vivo activities of phosphate derivatives of 9-(1,3-dihydroxy-2-propoxymethyl)-guanine against cytomegaloviruses", pages 299-308 see the whole document	1-15
X	Journal of Medicinal Chemistry, volume 29, no. 5, May 1986, American Chemical Society, (US), E.J. Prisbe et al.: "Synthesis and antiherpes virus activity of phosphate and phosphonate derivatives of 9-[(1,3-dihydroxy-2-propoxy)methyl] guanine", pages 671-675 see the whole document cited in the application	1-15
X	EP, A, 0173624 (MERCK & CO.) 5 March 1986 see the whole document	1-15

* Special categories of cited documents: 10

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the International filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
6th April 1988	09 MAY 1988

International Searching Authority	Signature of Authorized Officer
EUROPEAN PATENT OFFICE	P.C.G. VAN DER PUTTEN

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
	cited in the application -- 	
X	WO, A, 84/04748 (SRI INTERNATIONAL) 6 December 1984 see the whole document	1-15

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on 26/04/88
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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A- 0173624	05-03-86	JP-A- 61057584	24-03-86
WO-A- 8404748	06-12-84	SE-A- 8500307	23-01-85
		NL-A- 8420134	01-04-85
		EP-A- 0145739	26-06-85
		GB-A,B 2150570	03-07-85
		DE-T- 3490262	28-11-85
		JP-T- 60501407	29-08-85